the specification fails to provide enablement for making an anti-plasmodium vaccine, citing *Ellis* for the proposition that there is a recognition in the art that it is uncertain whether a single protein derived from a pathogen is capable of eliciting protective immunity.

Applicant respectfully traverses.

Claim 50 recites an anti-plasmodium vaccine that is comprised of an immunogenic amount of isolated p42 polypeptide and either a QS-21 or ISA51 adjuvant wherein the p42 polypeptide is expressed by an insect cell containing a vector which encodes the polypeptide and wherein the polypeptide is comprised of specific fragments of *Plasmodium falciparum* surface protein gp195.

35 U.S.C. §112, first paragraph requires that a specification be commensurately enabling relative to the scope of the claims and the issue of enablement is thus "whether it would take undue experimentation for one ordinarily skilled in the art to produce" embodiments that fall within the scope of the claims beyond any embodiment that is adequately disclosed in the specification. *Ex Parte Kung*, 17 U.S.P.Q.2d 1545, 1547 (Bd. Pat. App. & Interf. 1989). In the examples and throughout the specification, Applicant has unequivocally demonstrated that an immunogenic amount of isolated p42 polypeptide of *Plasmodium falciparum* surface protein gp195 (*i.e.*, expressed by insect cells containing a vector which encodes p42) induces antibodies which strongly inhibit parasite growth. The making and using of the anti-plasmodium vaccine, as recited in the claims, is therefore unequivocally shown and established by the Applicant.

The antibodies disclosed and described in the specification are shown to be extensively crossreactive with different parasite strains and to strongly or completely inhibit the growth of both heterologous and homologous *Plasmodium falciparum* parasites. Applicant directs the Examiner's attention to Example 9 (pages 42-43) of the specification demonstrating that rabbits intramuscularly immunized with BVp42 (*e.g.*, a variant form of the natural gp195 p42 processing fragment) produce serum antibodies which bind to both

recombinant BVp42 (demonstrating immunogenicity) and the native gp195 (demonstrating cross-reactivity), as shown by ELISA (*e.g.*, vinyl plates coated with recombinant p42 and parasite gp195), parasitic indirect immunofluorescence (*e.g.*, acetone-fixed blood smears of schizonts and merozoites), and immunoblotting with parasite gp195.

BVp42 is demonstrated to be highly immunogenic (*see e.g.*, Table 5) and elicits antibody titers comparable to the antibody titers elicited from rabbits which have been immunized with the purified, parasite gp195. High ELISA titers were obtained late in the quaternary response for both BVp42 and purified parasite gp195. BVp42 was additionally shown to produce typical merozoite surface staining patterns as demonstrated by indirect immunofluorescence assays ("IFA"). The IFA titers obtained after the fourth immunization reached levels which exceeded the titer levels obtained by immunization with the purified, parasite gp195 immunogen.

The data and results provided in Example 10 on page 46, lines 1-19, demonstrate that the BVp42 immunogen is recognized by the antisera of various congenic strains of mice (*e.g.*, different H-2 haplotypes on a B10 background) which were immunized with purified, parasite gp195. All seven mice strains assessed were shown to produce anti-gp195 antibodies recognizing epitopes of BVp42, demonstrating that mice strains exhibiting diverse MHC haplotypes are capable of producing antibodies which recognize BVp42. The data and results provided in Example 11 (on page 46, lines 21-33 of the specification) show that anti-BVp42 antibodies react with both homologous (*e.g.*, FUP parasite isolate) and heterologous (*e.g.*, FVO parasite isolate) gp195 antigens as demonstrated by identical ELISA titers and binding curves obtained using anti-BVp42 antibodies with both the homologous and heterologous forms of gp195 immunogen. Similar results were obtained in IFA assays using FVO (*e.g.*, heterologous parasite) and FUP (*e.g.*, homologous parasite) merozoites.

The anti-BVp42 antibodies were additionally demonstrated to strongly or completely inhibit the *in vivo* growth of heterologous (*e.g.*, FVO and Hond-1) and homologous parasites (*e.g.*, FUP and Pf857) in a well-established primate model. The data and results provided in Example 12 (on page 48, lines 1-34 and page 49, line 1) illustrate that *Aotus* monkeys immunized with either QS-21 or ISA51 adjuvant formulations generated antibody responses

to BVp42 immunogen wherein a significant boosting of antibody titer was observed after the second immunization (*see e.g.,* Figs. 8C and 8D, respectively). In addition, all *Aotus* monkeys immunized with either BVp42/QS21 or BVp42/ISA51 continued to develop increased antibody levels with repeated immunization and achieved peak antibody titers after the fourth immunization. Test animal cell mediated immune responses induced by immunization with BVp42 immunogen were assessed by either antigen specific T-cell proliferation assays (*e.g., see* Fig. 9) or by measurement of cytokine producing cells in BVp42 immunogen-stimulated T-cell cultures (*e.g., see* Figs. 10A-B). The cytokine data demonstrate that the claimed BVp42/QS21 and BVp42/ISA51 formulations induce priming of both Th1 and Th2-like lymphocyte populations in immunized *Aotus* monkeys (page 50, lines 15-19).

The data and results provided in Example 13 on page 50, lines 26-28, demonstrate high levels of parasite growth inhibition (*e.g.,* 94.3% and 92.3%) in the test animal immunized with BVp42/ISA51 which displayed the highest level of protective immunity. The data and results provided in Example 15 on page 54, lines 16-21, moreover, demonstrate that the course of infection in three of the four test animals immunized with BVp42/QS21 was significantly reduced relative to control group animals (*see e.g.,* Fig.11B). Test animals were additionally shown to experience prolonged periods of controlled parasite multiplication. Following the parasite multiplication phase, one test animal was even demonstrated to <u>self-cure</u> from infection due to clearance of parasites from the peripheral blood (page 54, lines 19-21). In addition, two of the three test animals immunized with BVp42/ISA51 experienced a prolonged prepatent period (*e.g.,* relative to control animals) after which time parasitemia increased to moderate levels and was then controlled for significant periods (*see* Fig. 11D). For example, the test animal immunized with BVp42/ISA51 displaying the highest level of protective immunity exhibited no detectable parasitemia until 23 days after challenge. Subsequent to this time, parasitemia levels peaked, abruptly dropped to low levels, and then <u>disappeared entirely from the host peripheral circulation</u> (page 54, lines 30-35). One of the test animals immunized with BVp42/ISA51, moreover, remained healthy and vigorous throughout the infection period and exhibited no reduction in erythrocyte count (page 54, line 35; page 55, line 1).

Further, Table 11 (page 55) provides ELISA titers of test animals immunized with BVp42/QS21 or BVp42/ISA51 and challenged with three different *P. falciparum* solid phase immunogens (*e.g.*, recombinant FUP BVp42, recombinant FVO BVp42, and parasite derived FUP MSP1). The data in Table 11 indicate that test animals immunized with BVp42/QS21 produced significantly high titers to both homologous and heterologous immunogen. A high level of cross reactivity was additionally demonstrated between the antibodies produced against recombinant p42 MSP1 and antibodies generated against homologous MSP1 purified from parasite extracts.

Accordingly, the results and data provided by Applicant are clearly sufficient to enable the making and using of the anti-plasmodium <u>vaccines</u> which are recited in the claims. Applicant therefore respectfully requests that Examiner's rejection of claim 50 (and dependent claims 51-55) under 35 U.S.C. §112, first paragraph be withdrawn.

Claim Rejections - 35 USC §103 (*Holder* in view of *Soltysik* and *Saul*)

Claims 37 (and dependent claims 38, 48, and 49) and 50 (and dependent claims 51-55) are rejected under 35 U.S.C. §103(a) as being unpatentable over *Holder et al.* in view of *Soltysik et al.* and *Saul et al.*

Claim 37 recites a pharmaceutical composition for treating plasmodium parasitemia in mammals which is comprised of an isolated p42 polypeptide in combination with either a QS-21 or ISA51 adjuvant.

The Examiner argues that Applicant's invention would be obvious to the ordinarily skilled artisan in light of *Holder* in view of *Soltysik* and *Saul*. The Examiner alleges that *Holder* teaches the sequence of the *Plasmodium falciparum* merozoite major surface antigens (83K, 42K, and 19K), *Soltysik* describes the use of QS-21 as an immunologic adjuvant, and *Saul* teaches the administration of MSP-1 and Montanide ISA720 adjuvant to humans. The Examiner further argues that the *Holder* p42 polypeptide, in view of the *Soltysik* QS-21 immunologic adjuvant, and the *Saul* method of administration, render Applicant's invention unpatentable under 35 U.S.C. §103(a). The Examiner further states that *Saul* provides the motivation for combining p42 with adjuvant ISA51 because *Saul* teaches that immunogenic

compositions containing adjuvants like Montanide ISA720 elicit better immune responses than immunogenic compositions which contain more traditional types of adjuvants.

Applicant respectfully traverses the rejection.

Applicant relies on the arguments submitted in the Response filed on December 17, 2001. Applicant further points out that the art has tried and failed to select protective adjuvant formulations suitable for use with the recombinant p42 sequences which are recited in the claims. It is well established that secondary considerations, such as the failure of others, may be evaluated for assessing the circumstances surrounding the subject matter sought to be patented, as indicia of non-obviousness with respect to a 35 U.S.C. §103(a) rejection. *See e.g., Graham, et al. v. John Deere Company of Kansas City, et al.; Calmar, Inc. v. Cook Chemical Company; Colgate-Palmolive Company v. Same,* 148 USPQ 459 (US Sup.Ct. 1966); *American Bank Note Holographics, Inc. v. The Upper Deck Co.,* 45 USPQ2d 1732 (DC SDNY 1997). The evidence establishing that persons have attempted for years but failed to create an invention superior to the invention that is claimed, moreover, may be used to demonstrate that the claimed invention was not obvious to the skilled artisan at the time of invention. *See e.g., Panduit Corporation v. Dennision Manufacturing Co.,* 227 USPQ 337 (CAFC 1985).

Applicant maintains that the art demonstrates there is significant uncertainty in the field regarding which adjuvant works optimally in combination with the particular p42 amino acid sequences claimed by Applicant. Stowers, *et al., Infection and Immunity,* 69(3):1536-1546 (2001) demonstrates that a recombinant form of MSP1 based on the 42-kDa C-terminal processing fragment of the merozoite major surface protein 1 (*e.g.,* bvMSP1_{42}) elicits high antibody production and confers protection in the host only when the recombinant MSP1 vaccine is formulated in Freund's adjuvant. When the MSP1 vaccine was formulated in an alternate adjuvant (*e.g.,* MF59), antibody titers were not adequate to confer protection to the animal host (*see* abstract). Protection in the host is dependent upon the production of high antibody titers. One of the art-recognized problems identified in Stowers, therefore, is that not every adjuvant used in combination with an MSP1 vaccine is capable of eliciting high antibody titers in the animal host (page 1536).

For example, host animals immunized with an MSP1 vaccine formulated in Freund's adjuvant produced "16 times more antibody" than test animals immunized with the MSP1 vaccine formulated in MF59. Moreover, all of the MF59 test animals required treatment for parasitemia, while only one (out of the 7 tested) of the Freund's adjuvant animals required parasitemia treatment (page 1541). These results suggest that formulation of the MSP1 vaccine in MF59, rather than Freund's adjuvant, may actually abrogate the host protective response. Titers elicited by MSP1 vaccine formulated in MF59 were between 8 and 15 times lower than titers elicited by the MSP1 vaccine that was formulated in Freund's adjuvant, and challenge by the virulent *Plasmodium falciparum* FVO strain in the animal model requires a powerful adjuvant for eliciting high antibody titers (page 1545). Efficacy was demonstrated for Freund's adjuvant although not for MF59. Accordingly, the data disclosed in Stowers *et al.* demonstrates that the skilled artisan cannot determine, prior to actual testing, which adjuvants will function adequately in combination with a particular immunogen for eliciting a high antibody response sufficient to confer immunity to an animal host.

Further evidence of this art-recognized problem is found in Kumar, *et al.* where a recombinant 19 kDa portion of the MSP-1 p42 processing fragment containing cysteine-rich epidermal growth factor-like domains was demonstrated to confer protective immunity to an animal host only when used in combination with Freund's adjuvant. *See* Kumar, *et al., Infection and Immunity*, 68(4):2215-2223 (2000). Out of the seven Freund's adjuvant animals tested, only one animal was not protected against *Plasmodium falciparum* challenge (page 2222). Immunogen 19 kDa formulations with non-Freund's adjuvants, however (*e.g.*, CFA and Freund's incomplete adjuvant, Rehydragel alum, SBAS2, Monatanide ISA 720, lipid A in liposomes, polyphosphazine, or microspheres) failed to confer protective immunity to any of the animal hosts tested (page 2216). Additionally, in Burghaus, *et al.*, a recombinant 19 kDa MSP-1 immunogen incorporated into liposomes and adsorbed to alum similarly failed to protect animal hosts against *Plasmodium falciparum* challenge. *See* Burghaus, *et al., Infection and Immunity*, 64(9):3614-3619 (1996). Secreted recombinant p42 processing proteins of the merozoite major surface protein 1 (*e.g.,* FVO p42 and FVO p42 with deleted N-linked glycosylation sites) administered in combination with Freund's

adjuvant, however, were demonstrated to elicit protective immunity in animal hosts. *See* Stowers, *et al., Proc Natl Acad Sci.,* 99(1):339-344 (2001). Accordingly, the Examiner's broad assertion that the mere disclosure of QS-21 as a potential adjuvant in *Soltysik* renders Applicant's specific combination of antigen and adjuvant obvious (*e.g.,* in view of the *Holder* p42 polypeptide and the *Saul* method of administration), is negated by the relevant art in the field which teaches the unpredictability of adjuvant efficacy in combination with the p42 sequences recited in Applicant's claims.

In particular, the art demonstrates that adjuvants such as (1) MF59, (2) CFA and Freund's incomplete adjuvant, (3) Rehydragel alum, (4) SBAS2, (4) Monatanide ISA 720, (5) lipid A in liposomes, (6) polyphosphazine, (7) microspheres, or (8) adjuvants incorporated into liposomes and adsorbed to alum, may not elicit adequate protective immunity in test animals when administered in combination with all or part of the p42 sequences recited in the claims. In fact, the relevant art demonstrates the failure of others to create viable combinations of p42 sequences and adjuvants for conferring immunity to animal hosts. The mere disclosure of QS-21 as an adjuvant (*e.g.,* in *Soltysik)* without substantive experimental data and results which link efficacy of the adjuvant to administration with the specific p42 sequences recited in the claims, therefore, fails to render Applicant's invention obvious. As demonstrated by the art in the field, the efficacy of an adjuvant administered in combination with a specific immunogen cannot be predicted until after the empirical tests have been performed. None of the prior art references cited by the Examiner demonstrates the efficacy of QS-21 (much less of ISA51) in specific combination with the p42 sequences recited in the claims, for conferring immunity to an animal host. Accordingly, Applicant respectfully requests withdrawal of the Examiner's rejection under 35 U.S.C. §103(a) of claims 37 (and dependent claims 38, 48, and 49) and 50 (and dependent claims 51-55).

Claim Rejections - 35 USC §103 (*Holder* in view of *Murphy* and *Smith*)

Claim 37 (and dependent claims 39-47) is rejected under 35 U.S.C. §103(a) as being unpatentable over *Holder et al.* in view of *Murphy et al.* and *Smith et al.* The Examiner argues that the *Smith* teaching of a recombinant baculovirus expression vector capable of

expressing a selected gene in a host insect cell, and the *Murphy* teaching of a recombinantly produced p42 from the WEL *Plasmodium falciparum* strain (also in a host insect cell), render Applicant's pharmaceutical composition *prima facie* obvious under 35 U.S.C. §103.

Applicant respectfully traverses the rejection.

Initially, Applicant notes that Claim 50, rather than Claim 37, recites "the expression of a p42 polypeptide by an insect cell which contains a vector encoding the polypeptide."

Applicant relies on the arguments submitted in the Response filed on December 17, 2001 and the arguments previously recited herein. Applicant further notes that not one of the prior art references cited by the Examiner discloses or suggests an adjuvant, much less an adjuvant whose efficacy is specifically linked to administration with p42 sequences for conferring protective immunity to an animal host, as required by the claims. Accordingly, the prior art references cited by the Examiner fail to teach or disclose all of the limitations of Applicant's claims. The Examiner's rejection under 35 U.S.C. §103 of Claim 37 (and claims 39-47 which depend therefrom) should therefore be withdrawn.

Based upon the foregoing, it is submitted that Claims 37-55 are patentable over the art of record.

The Commissioner is authorized to charge any additional fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 50-2319 (Our Order No. A-67984-/RFT/TAL/NBC).

CONCLUSION

Applicants respectfully submit that the Claims are in condition for allowance. If, upon review, the Examiner feels there are additional outstanding issues, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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APPENDIX OF PENDING CLAIMS

37. A pharmaceutical composition for treating plasmodium parasitemia in a mammal, said composition comprising:

an isolated p42 polypeptide in combination with an adjuvant selected from the group consisting of QS-21 and ISA51 and mixtures thereof.

38. The pharmaceutical composition of Claim 37, further comprising a pharmaceutically acceptable carrier.

39. The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide is expressed by an insect cell which contains a vector that encodes said polypeptide, and wherein said polypeptide is more immunogenic in a mammalian host than is the same polypeptide expressed in yeast.

40. The pharmaceutical composition of Claim 39, wherein said insect cell is selected from the group consisting of *Spodoptera frugiperda*, *Spodoptera exiaua*, *Choristoneura fumiferana*, *Trichoplusia ni* and *Spodoptera littoralis*.

41. The pharmaceutical composition of Claim 39, wherein said isolated p42 polypeptide is a *Plasmodium falciparum* polypeptide.

42. The pharmaceutical composition of Claim 41, wherein said *Plasmodium falciparum* polypeptide is an allelic form selected from the group consisting of MAD, K1, and Wellcome.

43. The pharmaceutical composition of Claim 37, wherein the transmembrane domain of said isolated p42 polypeptide is deleted.

44. The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide is fused to a second polypeptide.

45. The pharmaceutical composition of Claim 44, wherein said second polypeptide is a leader sequence fused to the amino terminus of said isolated p42 polypeptide.

46. The pharmaceutical composition of Claim 39, wherein said vector is a baculovirus vector.

47. The pharmaceutical composition of Claim 39, wherein said mammalian host is a primate.

48. The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

49. The pharmaceutical composition of Claim 48, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:5; and

(e) combinations thereof.

50. An anti-plasmodium vaccine comprising an immunogenic amount of an isolated p42 polypeptide expressed by an insect cell which contains a vector that encodes said polypeptide, in combination with an adjuvant selected from the group consisting of QS21 and ISA51 and mixtures thereof,

wherein said isolated p42 polypeptide is more immunogenic in a mammalian host than is the same polypeptide expressed in yeast.

51. The vaccine of Claim 50, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

52. The vaccine of Claim 51, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

53. A method of inducing an anti-plasmodium immune response in a mammal comprising administering to said mammal the vaccine of Claims 50, 51 or 52.

54. The method of Claim 53, wherein said immune response substantially reduces plasmodium parasitemia in said mammal.

55. The method of Claim 53, wherein said mammal is a primate.